

# Evaluation of Two Live, Cold-Passaged, Temperature-Sensitive Respiratory Syncytial Virus Vaccines in Chimpanzees and in Human Adults, Infants, and Children

Ruth A. Karron, Peter F. Wright, James E. Crowe, Jr.,  
Mary Lou Clements-Mann, Juliette Thompson,  
Mamodikoe Makhene, Roberta Casey,  
and Brian R. Murphy

Center for Immunization Research, Department of International Health,  
School of Hygiene and Public Health, Departments of Pediatrics and  
Medicine, School of Medicine, The Johns Hopkins University, Baltimore,  
and Laboratory of Infectious Diseases, National Institute of Allergy and  
Infectious Diseases, National Institutes of Health, Bethesda, Maryland;  
Vanderbilt Vaccine Center, Department of Pediatrics, Vanderbilt  
University School of Medicine, Nashville, Tennessee

Two live-attenuated, cold-passaged (*cp*), temperature-sensitive (*ts*) candidate vaccines, designated *cpts530/1009* and *cpts248/955*, were attenuated, genetically stable, and immunogenic in chimpanzees and were highly attenuated for human adults. In respiratory syncytial virus (RSV)-seropositive children, *cpts530/1009* was more restricted in replication than *cpts248/955*. In seronegative children,  $10^4$  pfu of *cpts248/955* was insufficiently attenuated, and a high titer of vaccine virus was shed (mean peak titer,  $10^{4.4}$  pfu/mL), whereas  $10^4$  pfu of *cpts530/1009* was relatively attenuated and restricted in replication (mean peak titer,  $10^{2.0}$  pfu/mL). At a dose of  $10^5$  pfu, *cpts530/1009* was immunogenic in seronegative children (geometric mean titer of RSV neutralizing antibodies, 1:724). Transmission of either vaccine to seronegative placebo recipients occurred at a frequency of 20%–25%. Of importance, vaccine viruses recovered from chimpanzees and humans were *ts*. In contrast to previous studies, this study indicates that live attenuated RSV vaccines that are immunogenic and phenotypically stable can be developed. Additional studies are being conducted to identify a live RSV vaccine that is slightly more attenuated and less transmissible than *cpts530/1009*.

Respiratory syncytial virus (RSV) is the leading cause of viral respiratory illness in infants and children throughout the world (reviewed in [1]) and is an important cause of severe respiratory illness in the elderly [2] and in immunocompromised patients [3]. In the United States, RSV infections account for ~90,000 hospitalizations of children each year [4].

The importance of RSV as a respiratory pathogen makes RSV vaccine development a priority [5]. Since an effective vaccine will need to provide protective immunity against RSV-associated lower respiratory tract illness (LRI) in young infants, RSV immunization will need to be initiated in the first month of life. It may be difficult to immunize this population effectively for several reasons. Young infants may respond poorly to an RSV vaccine because of immunologic immaturity and because maternally derived antibody may interfere with the immune response to the vaccine [1, 6–8]. Infants will also need to be immunized with a vaccine that protects against the

antigenically divergent RSV subgroups A and B. Finally, it is likely that infants will need to be immunized several times to achieve a satisfactory level of immunity since even a single infection with wild type (wt) RSV does not completely protect against subsequent RSV-associated LRI [9, 10].

Efforts to produce a safe and effective RSV vaccine have been underway for >30 years. Early attempts yielded a formalin-inactivated vaccine that produced enhanced disease in some immunized RSV-naïve infants when they were naturally infected with wt RSV during the subsequent RSV season [11, 12]. More recently, an RSV fusion (F) subunit vaccine has been produced that may prove useful in the elderly [13] but is unlikely to be administered to young infants. Adenovirus and vaccinia virus recombinants containing the RSV F and attachment (G) glycoproteins have also been developed but were not sufficiently immunogenic in chimpanzees to warrant clinical evaluation [14–16].

Live RSV vaccines might provide the best alternative for immunizing young infants. A live vaccine would mimic natural infection, induce a balanced cellular and humoral immune response, and be unlikely to produce enhanced disease [6]. In addition, live virus vaccines can replicate at mucosal surfaces even in the presence of passively acquired antibodies [7, 8, 17]. Previous live RSV candidate vaccines were either over- or underattenuated in young children [18–21], and those that were temperature-sensitive (*ts*) did not retain this phenotype during replication in vivo [22].

Recently, a series of live attenuated candidate vaccines was derived by chemical mutagenesis of an incompletely attenuated cold-passaged RSV mutant (*cp*RSV [23–25]). The parent virus,

Received 26 February 1997; revised 17 June 1997.

Guidelines for human experimentation of the Joint Committee for Clinical Investigation of the Johns Hopkins University School of Medicine and the Institutional Review Board of Vanderbilt University Medical Center were followed in the conduct of this study.

Financial support: NIH (AI-15095); Wyeth-Lederle Vaccines and Pediatrics.

Reprints or correspondence: Dr. Ruth A. Karron, Center for Immunization Research, Johns Hopkins University School of Hygiene and Public Health, Hampton House 117, 624 N. Broadway, Baltimore, MD 21205.

The Journal of Infectious Diseases 1997;176:1428–36  
© 1997 by The University of Chicago. All rights reserved.  
0022-1899/97/7606-0003\$02.00

*cpRSV*, a non-*ts* strain, contained mutations that restricted viral replication in adults and RSV-seropositive children but was insufficiently attenuated for RSV-seronegative children [19]. *cpRSV* was subjected to two rounds of chemical mutagenesis, and *ts* mutant derivatives (referred to as *cpts*) were generated [23–25]. It was hoped that the non-*ts* attenuating mutations present in the *cpRSV* parent virus would act in concert with the *ts* mutations to yield attenuated, genetically stable vaccines. Several *cpts* RSV mutants were subsequently shown to be restricted in replication in BALB/c mice and in chimpanzees [23, 24]. The *ts* phenotype of two of these mutants was more stable than that present in the prototype live attenuated RSV vaccine virus, *ts*-1 [23]. Here we describe the preclinical and phase I evaluation of RSV *cpts*248/955 and *cpts*530/1009 in chimpanzees and adults and in RSV-seropositive and -seronegative infants and children. For purposes of comparison, wt RSV strain A2 and *cpRSV* were also evaluated in adult volunteers and chimpanzees.

## Materials and Methods

**Viruses.** The isolation and characterization of wt RSV strain A2, of *cpRSV*, and of the *ts* mutants 248/955 (shutoff temperature, 37°C) and 530/1009 (shutoff temperature, 36°C) have been described [24–27]. Each of these *ts* mutants was derived independently from *cpRSV* by serial mutagenesis with 5-fluorouracil in Vero cell monolayer cultures. The *ts* mutants were biologically cloned by three plaque-to-plaque passages and amplified by four passages to prepare the vaccine pools. The wt RSV strain A2 (designated lot RSV M6) was prepared in MRC-5 cell monolayer cultures, and each of the progeny strains (*cpRSV*, lot A-11; *cpts*248/955, lot A-10; and *cpts*530/1009, lot A-16) were prepared in qualified Vero cell monolayer cultures. The *cpRSV* lot A-11 differed from the previously evaluated *cpRSV* [27] in that it was biologically cloned by three plaque-to-plaque passages in MRC-5 cell monolayer cultures. This cloned preparation of *cpRSV* served as the immediate parent for the *cpts* derivative viruses. Virus suspensions for clinical trials were produced and found to be free of adventitious agents by Louis Potash (Dyncorp/PRI, Bethesda, MD). The titers of the wt RSV A2 strain and of *cpRSV*, *cpts*248/955, and *cpts*530/1009 were  $10^{3.9}$ ,  $10^{5.8}$ ,  $10^{5.9}$ , and  $10^{5.5}$  pfu/mL, respectively. When necessary, the virus suspensions were diluted in L-15 medium (BioWhittaker, Walkersville, MD) immediately prior to use.

**Studies in chimpanzees.** Young male or female chimpanzees (*Pan troglodytes*) weighing 8.8–10.4 kg were pair-housed in large glass isolator suites and maintained as described previously [28]. The animals given *cpts*248/955 were on loan from the University of Texas MD Anderson Cancer Center (Bastrop). These chimpanzees lacked detectable serum neutralizing antibodies to RSV A2 (titer <1:10). Four seronegative chimpanzees were inoculated with *cpts*248/955 by both the intranasal and intratracheal routes with a dose of  $10^4$  pfu in a 1-mL inoculum at each site. Data from similar animals that received wt RSV strain A2, *cpRSV*, and *cpts*530/1009 were described previously [23, 25] and are presented here for the purpose of comparison. The comparability of the studies

was insured by the use of identical protocols and challenge virus suspensions. In addition, inoculation, sampling, and clinical scoring procedures were performed by the same individuals in each study. Following inoculation of virus, nasopharyngeal swab specimens were collected while animals were under ketamine anesthesia for quantitation of the amount of virus shed on days 1–10, 13, 16, and 20, and tracheal lavage specimens were collected on days 2, 4, 6, 8, 10, 13, 16, and 20, as described previously [15]. Virus present in the respiratory tract secretions was quantified by plaque titration at 32°C, 39°C, and 40°C on HEp-2 cells as previously described. The amount of rhinorrhea was estimated daily and assigned a score of 0 to 4 by an experienced observer (0 = none, 1 = trace, 2 = mild, 3 = moderate, 4 = severe [29]). One month after immunization, animals were challenged with wt RSV A2 virus as previously described [25].

**Clinical studies of adults.** The RSV A2 wt virus, *cpRSV*, *cpts*248/955, and *cpts*530/1009 were each evaluated in open-label, nonrandomized trials in healthy adults 18–45 years of age who were not in contact with immunosuppressed individuals or infants <1 year of age. Ninety-nine adults participated in these studies: 44 received the RSV wt A2 virus, 20 each received the *cpRSV* or the *cpts*248/955 viruses, and 15 received *cpts*530/1009. The health of the adult volunteers was assessed as previously described [29].

The wt RSV A2 virus was evaluated in the Johns Hopkins University Center for Immunization Research (CIR) isolation unit. Volunteers were given  $10^{3.9}$  pfu of RSV A2 wt virus in 1 mL intranasally. Nasal washes to quantitate virus shedding were performed daily, once prior to inoculation and for 10 days after. Volunteers were examined each day, and their temperatures and vital signs were recorded every 6 h during the 13-day isolation period. Vaccine strains were evaluated in outpatient studies at the CIR. Volunteers who received  $10^5$  pfu of *cpRSV*, *cpts*248/955, or *cpts*530/1009 intranasally were examined on the day of inoculation and on days 4–8 following inoculation. On each study day (0–10), volunteers recorded their own oral temperatures twice and reported any respiratory or febrile illness to the study nurse. All subjects who reported illness were examined by a study investigator.

**Clinical studies of children.** After *cpts*248/955 and *cpts*530/1009 were shown to be well tolerated in adults, these strains were evaluated in randomized, double-blind, placebo-controlled phase I trials in infants and children 6–60 months of age at the CIR and at the Vanderbilt University Vaccine Center (VVC). Ninety children were enrolled in these phase I safety and immunogenicity studies: 40 participated in studies of *cpts*248/955 and 50 in studies of *cpts*530/1009. Children were eligible to participate in these studies if they were healthy and if all other household members and day care contacts were  $\geq 1$  year of age. Prior to enrollment, children were screened for level of RSV serum neutralizing antibody by a complement-enhanced, 60% plaque-reduction neutralization assay [30]; those with titers >1:40 were considered RSV-seropositive. Both vaccines were initially tested at a dose of  $10^5$  pfu in seropositive children and tested subsequently at a dose of  $10^4$  pfu (*cpts*248/955 strain) or at  $10^4$  or  $10^5$  pfu (*cpts*530/1009 strain) in seronegative children. Each subject received 0.5 mL of vaccine or placebo intranasally. In the pediatric studies, the ratio of vaccinees to placebo recipients was ~2:1. Seropositive study participants were examined 2 days before inoculation and for 9 days after. Seronegative study participants were examined 2 days

before and on days 1–9, 11, 14, 16, 18, 21, and 23 after inoculation; interval symptom histories were obtained from parents on days when the children were not examined. Children were observed for 1–2 h at the CIR and for 6–10 h at the VVC in a playroom setting on each study day. Respiratory and febrile illnesses were defined as fever (rectal temperature,  $\geq 38.1^{\circ}\text{C}$ ), upper respiratory tract illness (URI; rhinorrhea or pharyngitis for  $\geq 2$  days), LRI (persistent wheezing or pneumonia), and cough (on  $\geq 2$  consecutive days) [31].

To assess the long-term safety of the *cpts* 248/955 and 530/1009 candidate vaccines, seronegative infants and children enrolled in these trials were followed through the subsequent RSV season. A group of RSV-seronegative children who did not receive vaccine or placebo were recruited as additional control subjects for this phase of the study. Children who participated in surveillance were monitored throughout the RSV season for fever and respiratory illnesses (as defined above). Nasal washes from ill children were tested for RSV by culture and by EIA (Testpack; Abbott Laboratories, Abbott Park, IL).

**Isolation, quantitation, identification, and phenotypic characterization of virus.** Nasal wash specimens for virus isolation were obtained on each day of observation from all subjects who participated in these studies. Fresh undiluted nasal wash specimens were titrated by plaque assay on HEp-2 cell monolayer cultures maintained under a semisolid overlay at  $32^{\circ}\text{C}$  as previously described, and results were expressed as  $\log_{10}$  pfu/mL [25]. Nasal wash samples were also inoculated into tubes containing Vero and HEp-2 cell culture monolayers. Virus isolates from these tubes were identified as RSV using an indirect IFA (Bartels Microscan; Baxter Healthcare, Bellevue, WA). For purposes of calculation, samples in which virus was not detected or did not produce plaques were assigned an infectivity titer of  $10^{0.6}$  pfu/mL.

**Phenotypic characterization of virus isolates.** To assess the stability of the *ts* phenotype, fresh nasal wash specimens from subjects who participated in trials of *cpts* 248/955 and *cpts* 530/1009 were titrated at  $32^{\circ}\text{C}$  and  $40^{\circ}\text{C}$ , and a more extensive analysis of efficiency of plaque formation at  $32^{\circ}\text{C}$ ,  $38^{\circ}\text{C}$ ,  $39^{\circ}\text{C}$ , and  $40^{\circ}\text{C}$  was determined subsequently using frozen aliquots of nasal wash specimens as previously described [23–25].

**Immunologic assays.** Sera and nasal wash specimens for measurement of RSV-specific antibodies were obtained from adults and RSV-seropositive children before and 4 weeks after inoculation and from RSV-seronegative children before and 8 weeks after inoculation. Sera were tested for antibodies to RSV by plaque reduction neutralization assay and for IgG antibodies to RSV F and G glycoproteins by end-point titration in an ELISA using immunoaffinity-purified F and G glycoproteins from RSV A2 infected cell lysates [32, 33].

Nasal wash samples were also tested for the presence of IgA antibody to purified RSV F and G glycoproteins by ELISA. Each ELISA nasal wash anti-RSV F or G IgA titer was corrected to a total IgA concentration of 100 mg/mL as measured by a radial immunodiffusion assay (Binding Site, San Diego) as previously described [29].

**Data analysis.** Laboratory evidence of infection with an RSV wt or vaccine strain was defined as isolation of RSV, a  $\geq 4$ -fold rise in serum RSV neutralizing antibody titer, and/or a  $\geq 4$ -fold rise in serum IgG antibody titer to purified RSV F and/or G glycoproteins. In several persons, isolated serum responses to either

RSV F or G glycoprotein were detected. In these instances, the ELISAs for both F and G glycoproteins were repeated and, if the response was confirmed, these individuals were considered to have been infected with the candidate vaccine virus. Isolated nasal wash antibody responses to RSV F or G glycoprotein were not considered definitive evidence of infection with vaccine virus.

RSV antibody titers were expressed as reciprocal mean  $\log_2$ . The Mann-Whitney *U* test (two-tailed) was used to compare mean titers between groups. Rates of illness among vaccinees and placebo recipients were compared by two-tailed Fisher's exact test.

## Results

**Response of RSV-seronegative chimpanzees to wt RSV A2, *cpRSV*, *cpts* 248/955, or *cpts* 530/1009.** Of the viruses studied, *cpts* 530/1009 was the most restricted in replication in the upper and lower respiratory tracts of seronegative chimpanzees (table 1). Of importance, the replication of both *cpts* 248/955 and 530/1009 was highly restricted in the lower respiratory tract, suggesting that it would be safe to evaluate these vaccines in clinical trials. RSV *cpts* 248/955 recovered from nasopharyngeal specimens ( $n = 30$ ) and tracheal lavage specimens ( $n = 5$ ) failed to produce plaques at  $40^{\circ}\text{C}$ , indicating that the *cpts* 248/955 candidate vaccine, like the previously evaluated *cpts* 530/1009 candidate vaccine, retained the *ts* phenotype after replication in seronegative chimpanzees [25]. The 4 chimpanzees that received the *cpts* 248/955 candidate vaccine developed a moderate titer of neutralizing antibody (geometric mean titer, 1:478) and, like the chimpanzees that received the *cpts* 530/1009 candidate vaccine, were completely resistant to challenge with wt RSV A2 virus [25].

**Response of adult volunteers to wt RSV A2, *cpRSV*, *cpts* 248/955, or *cpts* 530/1009.** *cpRSV*, *cpts* 248/955, and *cpts* 530/1009 replicated less well than wt virus in healthy adults (table 2). The 3 mutant viruses were less infectious than wt virus and were shed less frequently in these subjects ( $P = .01$ ,  $.003$ , and  $.001$  for *cpRSV*, *cpts* 248/955, and *cpts* 530/1009, respectively; Fisher's exact test). Respiratory, febrile, or systemic illnesses also occurred less often in recipients of these attenuated strains than in recipients of wt RSV A2 ( $P = .006$ ,  $<.001$ , and  $<.001$  for *cpRSV*, *cpts* 248/955, and *cpts* 530/1009, respectively; Fisher's exact test). Serum or nasal wash antibody responses to wt virus or vaccine were observed in about one-third of the study participants; the rate of response did not differ significantly between any of these groups (table 3). The attenuation of the *cpts* 248/955 and 530/1009 candidate vaccines for healthy adults led us to evaluate these *cpts* viruses in RSV-seropositive children. The biologically cloned *cpRSV* was not evaluated in children because previous studies indicated that uncloned *cpRSV* was insufficiently attenuated in RSV-seronegative infants [19].

**Response of RSV-seropositive children to *cpts* 248/955 or *cpts* 530/1009.** The *cpts* 248/955 and *cpts* 530/1009 candidate vaccines were evaluated at a dose of  $10^4$  or  $10^5$  pfu in seroposi-

**Table 1.** Response of RSV-seronegative chimpanzees to intranasal and intratracheal infection with  $10^4$  pfu of wild type RSV A2, *cp*RSV, A2 *cpts*248/955, or *cpts*530/1009.

RSV administered	No. of chimpanzees	No. infected	Rhinorrhea score mean (SD)	Mean peak titer* (SD) of virus in	
				Nasopharynx	Trachea
Wild type	4	4	1.4 (0.9)	5.5 (0.4)	5.7 (0.3)
<i>cp</i> RSV	2	2	0.6 (0.1)	4.6 (0.5)	2.9 (0.1)
<i>cpts</i> 248/955	4	4	0.9 (0.2)	4.6 (0.8)	1.6 (1.6)
<i>cpts</i> 530/1009	4	4	0.5 (0.3)	3.6 (0.5)	1.0 (0.6)

NOTE. For purposes of calculation, titer of 0.7 pfu/mL was assigned to culture-negative samples.

\* Virus titers are expressed as  $\log_{10}$  pfu/mL.

tive children (table 2). *cpts*248/955 infected the majority of vaccinees at each dose tested, and children who received  $10^5$  pfu of this candidate vaccine shed virus in titers as high as  $10^{4.7}$  pfu/mL (mean,  $10^{2.7}$ ). In contrast, *cpts*530/1009 infected few vaccinees and was not recovered from any seropositive child, indicating that it was more attenuated than *cpts*248/955 for seropositive children. The absence of LRI in seropositive recipients of the *cpts*248/955 and 530/1009 candidate vaccines suggested that it was safe to continue the evaluation of these *cpts* viruses in RSV-seronegative children.

The local and systemic immune responses of seropositive children to each of these candidate vaccine viruses are shown in table 3. At the  $10^5$ -pfu dose, a serum neutralizing or glycoprotein ELISA antibody response was observed in 62% of the vaccinees who received *cpts*248/955 and 31% of those who received *cpts*530/1009. Nasal antibody responses to either candidate vaccine were detected less frequently in these seropositive children. A single placebo recipient in the *cpts*248/955 vaccine study developed a 4-fold rise in serum antibody titer to the RSV F glycoprotein, which might have

**Table 2.** Clinical and virologic responses of adults and seropositive and seronegative children to wild type RSV A2, *cp*RSV, *cpts*248/955, *cpts*530/1009, or placebo.

Subjects	Virus given	Dose ( $\log_{10}$ pfu)	No. of subjects	% infected	Virus isolation (nasal wash)			% with indicated illness					
					% shedding	Duration of shedding, mean (SD)	Peak titer, mean (SD) $\log_{10}$ pfu/mL	Fever	URI	LRI	Cough	Otitis media	Any RSV-like illness
Adults	Wild type	3.9	44	50	43*†‡	6.8 (2.8)	3.3 (1.5)	7	41	7	0	0	52 <sup>§  †</sup>
	<i>cp</i> RSV	5.0	20	30	10*	2.7 (3.8)	1.7 (2.1)	5	10	0	0	5	15 <sup>§</sup>
	248/955	5.0	20	10	5 <sup>†</sup>	4.0 (4.0)	2.8 (2.2)	0	5	0	0	0	5 <sup>  </sup>
	530/1009	5.0	15	33	0 <sup>‡</sup>	0	$\leq 0.6$	0	0	0	0	0	0 <sup>†</sup>
Children	Seropositive	248/955	6	67	17	5.3 (9.1)	1.1 (0.9)	67	67	0	0	17	100
		530/1009	5	0	0	0	$\leq 0.6$	40	0	0	20	0	60
		248/955	13	62	38	5.1 (4.0)	2.7 (1.8)	15	7	0	0	0	23
		530/1009	13	31	0	0	$\leq 0.6$	15	0	0	0	0	15
		Placebo <sup>††</sup>	9	0	0	0	$\leq 0.6$	44	0	0	0	11	55
		Placebo <sup>‡‡</sup>	7	0	0	0	$\leq 0.6$	14	0	0	0	0	14
	Seronegative	248/955	8	88	88	9.0 (1.9)	4.4 (1.0)	88	88	12	25	25	100
		530/1009	7	86	43	4.8 (5.7)	2.0 (1.5)	57	71	0	0	14	71
		530/1009	8	100	100	11.0 (1.7)	4.5 (1.8)	62	62	12	0	38	88
		Placebo <sup>††</sup>	4	25	25	23	3.7	75	100	0	25	0	100
		Placebo <sup>‡‡</sup>	10	20	10	8.0 (8.0)	1.2 (0.6)	40	60	10	0	20	80

NOTE. Six- to 60-month-old RSV-seropositive children and 6- to 36-month-old RSV-seronegative children were enrolled in these studies. For purposes of this study, seropositive children were those with RSV serum plaque-reduction neutralizing antibody titers  $> 1:40$ . Infection was defined as described in text. URI, upper respiratory tract illness; LRI, lower respiratory tract illness. Duration of shedding is defined as last day on which vaccine virus was recovered.

\*  $P = .01$ , <sup>†</sup> .003, <sup>‡</sup> .001; <sup>§</sup> .006; <sup>||</sup>  $< .001$ ; <sup>††</sup>  $< .001$ .†† = placebo recipients in studies of RSV A2 *cpts*248/955 virus.‡‡ = placebo recipients in studies of RSV A2 *cpts*530/1009 virus.

**Table 3.** Immunologic responses of adults and RSV-seropositive and -seronegative children to wild type RSV A2, *cp*RSV, *cpts248/955*, *cpts530/1009*, or placebo.

Subjects, virus given	Dose (log <sub>10</sub> pfu)	n	% infected	% with serum antibody response	Mean RSV serum NA titer			Mean RSV serum ELISA titer (SD)						Mean nasal wash ELISA titer (SD)					
					(SD)			F glycoprotein			G glycoprotein			F glycoprotein			G glycoprotein		
					Before	After	% with ≥4-fold rise	Before	After	% with ≥4-fold rise	Before	After	% with ≥4-fold rise	Before	After	% with ≥4-fold rise	Before	After	% with ≥4-fold rise
Adults																			
Wild type	3.9	44	50	39	NT	NT	—	10.8 (1.7)	10.9 (1.6)	16	10.4 (1.7)	10.9 (1.7)	30	4.6 (1.7)	5.5 (2.1)	36	4.6 (2.1)	6.0 (2.3)	36
cpRSV	5.0	20	30	30	10.0 (1.3)	9.9 (1.6)	0	10.4 (1.3)	10.4 (1.2)	15	9.7 (1.4)	10.1 (1.5)	20	4.7 (2.1)	5.7 (1.8)	15	4.4 (1.9)	5.2 (2.4)	30
248/955	5.0	20	10	10	10.0 (1.0)	9.9 (1.0)	5	10.6 (1.3)	10.6 (1.3)	5	10.1 (1.2)	9.9 (1.3)	0	4.5 (1.5)	4.2 (1.6)	5	5.0 (1.6)	5.2 (2.0)	10
530/1009	5.0	15	33	33	9.9 (1.1)	10.4 (1.0)	7	10.4 (1.4)	11.0 (1.4)	27	9.8 (1.9)	9.8 (1.4)	20	4.7 (2.1)	5.1 (1.7)	20	5.2 (2.4)	6.1 (2.1)	27
Children																			
Seropositive																			
248/955	4.0	6	67	67	8.9 (1.0)	10.3 (0.6)	33	9.0 (2.1)	10.0 (0.9)	50	7.0 (2.1)	7.0 (2.1)	0	3.2 (0.7)	5.1 (1.2)	17	2.4 (1.2)	4.3 (1.9)	17
530/1009	4.0	5	0	0	7.3 (2.3)	7.5 (2.4)	0	10.9 (0.8)	10.5 (1.0)	0	9.3 (2.8)	8.5 (2.0)	0	4.2 (1.3)	3.1 (1.1)	0	4.2 (0.7)	3.1 (0.3)	0
248/955	5.0	13	62	62	9.2 (1.3)	9.9 (1.0)	8	11.0 (1.7)	11.0 (2.1)	31	8.2 (1.2)	9.5 (1.5)	54	4.5 (2.5)	5.7 (2.3)	15	3.0 (2.0)	3.9 (2.0)	8
530/1009	5.0	13	31	31	10.0 (1.0)	10.4 (0.8)	15	10.1 (1.7)	10.5 (1.5)	31	7.5 (1.8)	7.9 (2.1)	31	4.6 (1.8)	4.6 (1.6)	8	3.0 (1.4)	2.7 (1.2)	0
Placebo*	0.0	9	11	11	9.1 (1.3)	8.9 (1.3)	0	11.2 (2.0)	10.7 (1.3)	11	8.7 (1.2)	8.3 (1.0)	0	3.6 (2.2)	5.1 (1.9)	0	2.6 (1.6)	3.8 (2.6)	11
Placebo†	0.0	7	0	0	9.1 (2.7)	9.0 (2.7)	0	9.6 (2.9)	9.0 (2.9)	0	7.0 (2.3)	6.4 (2.1)	0	5.2 (1.9)	4.7 (2.0)	0	2.9 (1.3)	2.9 (0.9)	0
Seronegative																			
248/955	4.0	8	88	88	≤4.6 (0.0)	9.6 (2.1)	88	6.6 (2.0)	9.1 (1.9)	75	6.6 (1.4)	8.3 (1.7)	62	3.8 (2.6)	3.9 (1.3)	25	3.8 (2.6)	4.2 (1.5)	25
530/1009	4.0	7	86	86	≤4.6 (0.0)	8.1 (2.7)	57	7.0 (2.2)	8.3 (1.5)	57	6.2 (1.8)	7.0 (1.4)	57	2.0 (2.0)	4.3 (1.6)	43	1.8 (1.8)	3.1 (2.3)	28
530/1009	5.0	8	100	100	≤4.6 (0.0)	9.5 (0.6)	100	7.6 (2.1)	8.8 (2.2)	88	7.8 (1.9)	10.3 (1.0)	75	4.9 (1.3)	4.6 (3.2)	12	5.1 (1.3)	5.6 (2.8)	25
Placebo*	0.0	4	25	25	≤4.6 (0.0)	5.8 (1.3)	25	5.3 (1.4)	5.8 (1.7)	25	5.8 (1.7)	5.8 (1.7)	25	2.7 (1.7)	1.8 (0.6)	0	2.7 (1.7)	2.1 (1.0)	25
Placebo†	0.0	10	20	20	≤4.6 (0.0)	5.3 (2.0)	10	6.7 (2.5)	6.3 (2.0)	20	6.9 (2.2)	6.6 (1.4)	20	2.3 (2.1)	3.6 (2.6)	10	2.0 (1.3)	2.6 (2.0)	0

NOTE. All titers are expressed as mean reciprocal log<sub>2</sub>. Nasal wash antibody titers were corrected to 100 mg/mL total IgA. For purposes of calculation, nasal wash antibody titers ≤0 were assigned value of 0.1. NT, not tested; NA, neutralizing antibody.

\* Placebo recipients in studies of RSV A2 *cpts248/955* virus.

† Placebo recipients in studies of RSV A2 *cpts530/1009* virus.

resulted from transmission of vaccine virus from an infected vaccinee.

**Response of RSV-seronegative children to *cpts248/955* or *cpts530/1009* vaccines.** At a dose of 10<sup>4</sup> pfu, *cpts248/955* was infectious and immunogenic but not sufficiently attenuated for RSV-seronegative infants and children (table 2). Eighty-eight percent of seronegative infants and children shed vaccine virus (mean peak titer, 10<sup>4.4</sup> pfu/mL). Respiratory or febrile illness or otitis media associated with shedding of *cpts248/955* was observed in all infected children, 1 of whom also had a viral enanthem consistent with enterovirus infection. One child who received *cpts248/955* had 3 days of LRI (wheezing) associated with viral shedding (figure 1). This child, who was treated as an outpatient, received nebulized bronchodilators and oral bronchodilators and steroids and recovered uneventfully. Because the *cpts248/955* candidate vaccine retained the capacity to cause LRI, its clinical evaluation was terminated. This virus was also transmitted to a placebo recipient, who shed virus (10<sup>3.7</sup> pfu/mL) and had rhinorrhea and cough on days 21–23.

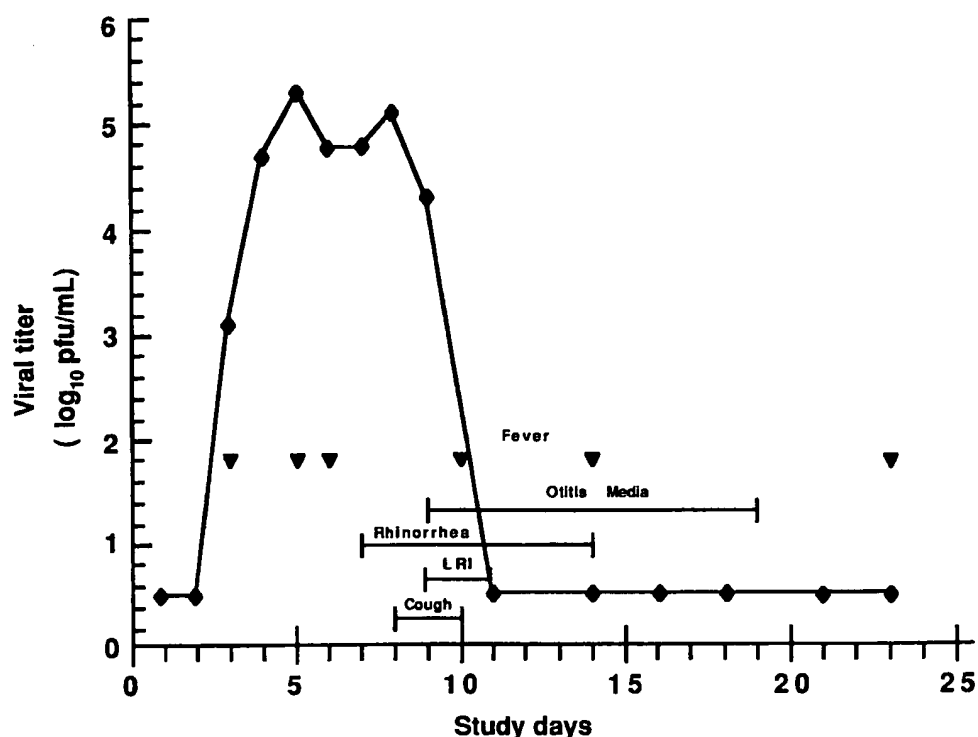
At a dose of 10<sup>4</sup> pfu, RSV *cpts530/1009* was more attenuated than *cpts248/955*. The mean peak titer of virus shed by seronegative vaccinees was 10<sup>2.0</sup> pfu/mL, which was less than that shed by seronegative recipients of the *cpts248/955* candidate vaccine ( $P = .01$ , Mann-Whitney  $U$  test). LRI was

not observed in children who received 10<sup>4</sup> pfu of the *cpts530/1009* candidate vaccine. At a dose of 10<sup>5</sup> pfu, clinical evaluation of *cpts530/1009* was complicated by simultaneous infection with adenovirus in 3 vaccinees and 1 placebo recipient. LRI was observed in 1 vaccinee and 1 placebo recipient, who both shed adenovirus and *cpts530/1009*, but was not observed in other study participants. The *cpts530/1009* mutant was apparently transmitted to this placebo recipient, who shed 10<sup>1.7</sup> pfu of vaccine virus on a single day (day 16) that did not coincide with her LRI (days 18–20). URI, low-grade fever, and otitis media occurred frequently but at approximately the same rate in vaccinees and placebo recipients (table 2).

The *cpts248/955* and 530/1009 candidate vaccines were highly immunogenic in RSV-seronegative children (table 3). Rises in neutralizing or F or G antibody titers were detected in 88% of those who received 10<sup>4</sup> pfu of *cpts248/955* and all of those who received 10<sup>5</sup> pfu of *cpts530/1009*. Nasal antibody responses were detected less frequently than serum antibody responses in these children, perhaps because of the insensitivity of the assay as compared with that of serologic assays.

A total of 23 seronegative vaccinees (8 of whom received *cpts248/955* and 15 of whom received *cpts530/1009*) and 64 unvaccinated children (13 placebo recipients and 51 control

**Figure 1.** Response of seronegative vaccinee to  $10^4$  pfu of RSV A2 *cpts248/955* vaccine.



subjects) participated in RSV surveillance. Twelve (52%) of the vaccinees and 31 (48%) of the unvaccinated subjects were infected with wt RSV during surveillance. Of the infected vaccinees, all had URI, 2 had fever, 2 had otitis media, and one had LRI. The single subject with LRI (wheezing and crackles on auscultation) was a recipient of *cpts530/1009* who did not shed vaccine virus but had developed a neutralizing antibody response to RSV. Of the infected unvaccinated subjects, all had URI, 11 had fever, 8 had otitis media, and 3 had LRI (all 3 children wheezed; 1 also had crackles on auscultation). Thus, there was no evidence of enhanced disease when recipients of these live RSV candidate vaccines were infected with wt RSV.

**Phenotypic stability of the *cpts248/955* and *530/1009* vaccines.** Despite a moderate to high level of replication in the upper respiratory tracts of RSV-seropositive and -seronegative children, the *cpts248/955* candidate vaccine retained the *ts* phenotype: Each of 40 nasal wash specimens containing virus produced plaques at 32°C but not at 40°C. Similarly, virus present in each of 71 specimens from vaccinees infected with the *cpts530/1009* candidate vaccine retained the *ts* phenotype. The efficiency of plaque formation of virus present in the nasal washes of 5 *cpts530/1009* vaccinees (table 4) indicated that little drift in the level of temperature sensitivity occurred during viral replication in fully susceptible children over an interval of 9–14 days.

## Discussion

The strategy of passaging virus at low temperature to yield mutants that replicate efficiently at suboptimal temperature but

are restricted in replication at core human body temperature has been used successfully to generate cold-adapted (*ca*) *ts* vaccines against influenza virus and parainfluenza virus type 3 (reviewed in [34]), [35]. Earlier attempts to develop RSV vaccines that were either *cp* or *ts* were initially abandoned because of residual virulence for RSV-seronegative infants and because of the genetic instability of the RSV *ts*-1 mutant [1]. Recently, *cp*RSV was further mutagenized to generate a series of *cpts* vaccine candidates that were shown to be attenuated and genetically stable in mice and seronegative chimpanzees [15, 23–25]. The *cp*RSV evaluated in this study was a biologically cloned derivative of the *cp*RSV previously evaluated in the 1960s and 1970s. In the present study, we found that the cloned *cp*RSV, the immediate parent of the *cpts* vaccines, was attenuated in adults. This indicates that the biologically cloned *cp*RSV contains non-*ts* attenuating mutations that should be present in its *cpts* derivatives. Indeed, the *cpts* viruses sequenced to date contain the five nucleotide mutations [36] present in the biologically cloned *cp*RSV parent virus. Thus, one or more of these non-*ts* mutations attenuate *cp*RSV for chimpanzees and adults.

In this study, we also demonstrated that the *cp*RSV, *cpts248/955*, and *cpts530/1009* viruses showed a progressive increase in attenuation for seronegative chimpanzees and adults. However, the *cpts248/955* candidate vaccine was not sufficiently attenuated for fully susceptible (i.e., RSV-seronegative) children because it was shed in large quantities from the upper respiratory tract, temporally associated with rhinorrhea in 6 children and with LRI in 1 child, and transmitted to a placebo

**Table 4.** Characterization of the *ts* phenotype of virus recovered from 5 seronegative children who received 10<sup>5</sup> pfu of RSV A2 *cpts530/1009* vaccine.

Assay no.	Vaccinee no. or virus	Study day	Titer of virus in nasal aspirate (log <sub>10</sub> pfu/mL) at indicated temperature (°C)			
			32	38	39	40
1	26591	4	3.8	<0.7	<0.7	<0.7
		5	4.6	<0.7	<0.7	<0.7
		6	5.3	<0.7	<0.7	<0.7
		7	5.2	<0.7	<0.7	<0.7
		8	>6.5	3.6*	<0.7	<0.7
		9	4.5	1.1*	<0.7	<0.7
1	26878	11	4.6	<0.7	<0.7	<0.7
		6	4.7	<0.7	<0.7	<0.7
		7	5.5	<0.7	<0.7	<0.7
		8	4.5	<0.7	<0.7	<0.7
2	141	9	3.6	<0.7	<0.7	<0.7
		9	4.6	<0.7	<0.7	<0.7
		11	2.9	<0.7	<0.7	<0.7
3	589	14	1.0	<0.7	<0.7	<0.7
		3	1.3	<0.7	<0.7	<0.7
		4	3.0	<0.7	<0.7	<0.7
3	590	5	4.1	<0.7	<0.7	<0.7
		6	4.7	<0.7	<0.7	<0.7
		7	4.5	2.0*	<0.7	<0.7
		8	4.6	<0.7	<0.7	<0.7
		9	5.1	<0.7	<0.7	<0.7
		10	3.7	<0.7	<0.7	<0.7
		11	3.0	<0.7	<0.7	<0.7
		3	3.7	<0.7	<0.7	<0.7
		4	4.1	<0.7	<0.7	<0.7
		5	3.6	<0.7	<0.7	<0.7
		6	6.4	<0.7	<0.7	<0.7
1	<i>cpts530/1009</i>	7	5.9	2.0*	<0.7	<0.7
		8	3.7	<0.7	<0.7	<0.7
		9	5.5	<0.7	<0.7	<0.7
		10	5.8	<0.7	<0.7	<0.7
		11	4.5	<0.7	<0.7	<0.7
		—	5.9	3.5*	<0.7	<0.7
		—	5.2	<0.7	<0.7	<0.7
		—	5.9	<0.7	<0.7	<0.7
		—	>6.5	>6.5	>6.5	>6.2†
		—	>6.5	>6.5	>6.5	>6.5
		—	>6.5	>6.5	>6.5	>6.5

\* Pinpoint plaque phenotype (<10% wild type plaque size at 32°C).

† Small plaque phenotype (<50% wild type plaque size at 32°C).

recipient. These studies suggest that RSV candidate vaccines, such as *cpts248/955*, that replicate to high titer in seropositive children may not be sufficiently attenuated for seronegative children. Evaluation of the *cpts530/1009* candidate vaccine in seropositive children showed that this virus was more restricted in replication and more attenuated than *cpts248/955*. The clinical evaluation of *cpts530/1009* in seronegative children was complicated by concurrent adenovirus infection in 4 subjects (and LRI in 2 subjects infected with both viruses); however, LRI was only observed in the children who shed adenovirus,

which suggests that *cpts530/1009* may be more attenuated than *cpts248/955* in these susceptible children. Of importance, there was no evidence of disease enhancement when recipients of either of these live RSV candidate vaccines were infected with wt RSV.

The *cpts248/955* vaccine was highly attenuated for chimpanzees and yet was able to cause LRI in seronegative children. This was an unexpected finding, and the reasons for this difference in response are not known. However, the relative order of attenuation of these viruses in chimpanzees (wt A2 virus being the most virulent, followed sequentially by *cpRSV*, *cpts248/955*, and *cpts530/1009*) was identical to that observed in our clinical studies, indicating that preclinical evaluation in chimpanzees provides valuable information about live RSV A vaccines destined for evaluation in humans.

In seronegative children, the *cpts248/955* and *530/1009* candidate vaccines were highly infectious and immunogenic. The geometric mean titers of RSV neutralizing antibody achieved in recipients of 10<sup>4</sup> pfu of the 248/955 vaccine (1:776) and of 10<sup>5</sup> pfu of 530/1009 vaccine (1:724) were well above the level believed necessary to protect the lower respiratory tracts of susceptible infants [37]. It is hoped that similar levels of neutralizing antibodies might be induced in seronegative children with further attenuated *cpts* RSV vaccines, especially if more than one dose of vaccine is administered. These further attenuated *cpts* vaccines are, however, minimally infectious in adults and RSV-seropositive children, so it is likely that other vaccines will be needed to prevent serious RSV disease in the elderly and in RSV-seropositive children with chronic lung disease [2, 38].

The *cpts248/955* and *530/1009* candidate vaccines were each recovered from a single seronegative placebo recipient. This is not surprising, since wt RSV spreads rapidly through susceptible populations [1, 9], and previous studies of the RSV *ts*-1 candidate vaccine showed that *ts* virus was recovered from a study nurse and a placebo recipient [18, 22]. In addition, some of the seronegative vaccinees in our studies shed virus in titers as high as 10<sup>5.0</sup> pfu/mL, which would likely exceed the dose required to infect a susceptible contact. Of note, the vaccine virus recovered from placebo recipients retained the *ts* phenotype. It may be that a live attenuated RSV vaccine, like live poliovirus vaccines, will retain the ability to spread to contacts, and studies of future live RSV candidate vaccines will need to address this possibility.

The stability of the *ts* phenotype of the *cpts248/955* and *530/1009* candidate vaccines was assessed by determining the efficiency of plaque formation of virus present in 40 nasal wash specimens of those who received the 248/955 candidate vaccine and 71 nasal wash specimens of those who received the 530/1009 candidate vaccine. None of the virus present in the nasal washes produced plaques at 40°C, indicating that the *ts* phenotype was maintained despite vigorous replication of these viruses in the upper respiratory tracts of RSV-seronegative subjects. This is the first evidence that live RSV candidate vaccines

can be produced that maintain the *ts* phenotype after replication in seronegative children because previous studies of the RSV *ts*-1 vaccine in chimpanzees and young children showed that *ts*<sup>+</sup> revertant virus could be recovered from nasal wash specimens [18, 29]. It is not known why the *cpts* live RSV candidate vaccines have a more stable phenotype than RSV *ts* candidate vaccines. It may be that the stability of the *ts* phenotype in the *cpts* vaccines is augmented by additional non-*ts* attenuating mutations already present in the *cp* parent virus, as has been observed with cold-adapted influenza virus vaccines [39]. These findings are encouraging and provide the basis for continued development and evaluation of *cpts* candidate vaccines.

In summary, we have shown that live *cpts* RSV candidate vaccines can be produced that are phenotypically stable following replication in RSV-seronegative children. A further attenuated derivative of *cp*RSV, *cpts*248/404, has been developed [25], and this vaccine may prove to be more restricted in replication and less transmissible than *cpts*530/1009. Evaluation of the *cpts*248/404 vaccine is in progress.

#### Acknowledgments

We thank Barbara Burns, Kathleen Gay, Anna Speaks, Bhagvanji Thumar, Sharon Tollefson, Ellen Davis, Jean Froehlich, and Denos Harris for expert clinical and technical assistance, Martin Blair for assistance with manuscript preparation, and Robert Chanock for review of the manuscript.

#### References

- Collins PL, McIntosh K, Chanock RM. Respiratory syncytial virus. In: Fields BN, Knipe DM, Howley PM, eds. *Fields virology*. New York: Raven Press, 1996:1313–51.
- Falsey AR, Cunningham CK, Barker WH, et al. Respiratory syncytial virus and influenza A infections in the hospitalized elderly. *J Infect Dis* 1995;172:389–94.
- Hall CB, Powell KR, MacDonald NE, et al. Respiratory syncytial viral infection in children with compromised immune function. *N Engl J Med* 1986;315:77–81.
- Anderson LJ, Parker RA, Strikas RL. Association between respiratory syncytial virus outbreaks and lower respiratory tract deaths of infants and young children. *J Infect Dis* 1990;161:640–6.
- Heilman CA. Respiratory syncytial and parainfluenza viruses. *J Infect Dis* 1990;161:402–6.
- Murphy BR, Hall SL, Kulkarni AB. An update on approaches to the development of respiratory syncytial virus (RSV) and parainfluenza virus type 3 (PIV3) vaccines. *Virus Res* 1994;32:13–36.
- Clements ML, Makhene MK, Karron RA, et al. Effective immunization with live attenuated influenza A virus can be achieved in early infancy. *J Infect Dis* 1996;173:44–51.
- Karron RA, Steinhoff MC, Subbarao EK, et al. Safety and immunogenicity of a cold-adapted influenza A (H1N1) reassortant virus vaccine administered to infants under six months of age. *Pediatr Infect Dis J* 1995;14:10–6.
- Henderson FW, Collier AM, Clyde WA Jr, Denny FW. Respiratory syncytial virus infections, reinfections, and immunity: a prospective, longitudinal study in young children. *N Engl J Med* 1979;300:530–4.
- Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. *Am J Dis Child* 1986;140:543–6.
- Kapikian AZ, Mitchell RH, Chanock RM, Shvedoff RA, Stewart CE. An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. *Am J Epidemiol* 1969;89:405–21.
- Kim HW, Canchola JG, Brandt CD, Pyles G, Chanock RM, Jensen K. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. *Am J Epidemiol* 1969;89:422–34.
- Falsey AR, Walsh EE. Safety and immunogenicity of a respiratory syncytial virus subunit vaccine (PPF-2) in ambulatory adults over age 60. *Vaccine* 1996;14:1214–8.
- Collins PL, Purcell RH, London WT, Lawrence LA, Chanock RM, Murphy BR. Evaluation in chimpanzees of vaccinia virus recombinants that express the surface glycoproteins of human respiratory syncytial virus. *Vaccine* 1990;8:164–8.
- Crowe JE Jr, Collins PL, London WT, Chanock RM, Murphy BR. A comparison in chimpanzees of the immunogenicity and efficacy of live attenuated respiratory syncytial virus (RSV) temperature-sensitive mutant vaccines and vaccinia virus recombinants that express the surface glycoproteins of RSV. *Vaccine* 1993;11:1395–404.
- Hsu KHL, Lubeck MD, Davis AR, Bhat RA, Selling BH, Bhat BM. Immunogenicity of recombinant adenovirus–respiratory syncytial virus using Ad4, Ad5, and Ad7 vectors in dogs and a chimpanzee. *J Infect Dis* 1992;166:769–6.
- Karron RA, Makhene M, Gay K, Wilson MH, Clements ML, Murphy BR. Evaluation of a live attenuated bovine parainfluenza type 3 vaccine in 2-to-6-month-old infants. *Pediatr Infect Dis J* 1996;15:655–61.
- Kim HW, Arrobio JO, Brandt CD, et al. Safety and antigenicity of temperature-sensitive (*ts*) mutant respiratory syncytial virus (RSV) in infants and children. *Pediatrics* 1973;52:56–63.
- Kim HW, Arrobio JO, Pyles G, et al. Clinical and immunological response of infants and children to administration of low-temperature adapted respiratory syncytial virus. *Pediatrics* 1971;48:745–55.
- Wright PF, Belshe RB, Kim HW, VanVorhis LP, Chanock RM. Administration of a highly attenuated, live respiratory syncytial virus vaccine to adults and children. *Infect Immun* 1982;37:397–400.
- Wright PF, Shinozaki T, Fleet W, Sell SH, Thompson J, Karzon DT. Evaluation of a live, attenuated respiratory syncytial virus vaccine in infants. *J Pediatr* 1976;88:931–9.
- McIntosh K, Arbeter AM, Stahl MK, Orr IA, Hodes DS, Ellis EC. Attenuated respiratory syncytial virus vaccines in asthmatic children. *Pediatr Res* 1974;8:689–96.
- Crowe JE Jr, Bui PT, London WT, et al. Satisfactorily attenuated and protective mutants derived from a partially attenuated cold-passaged respiratory syncytial virus mutant by introduction of additional attenuating mutations during chemical mutagenesis. *Vaccine* 1994;12:691–9.
- Crowe JE Jr, Bui PT, Davis AR, Chanock RM, Murphy BR. A further attenuated derivative of a cold-passaged temperature-sensitive mutant of human respiratory syncytial virus (RSV *cpts*-248) retains immunogenicity and protective efficacy against wild-type challenge in seronegative chimpanzees. *Vaccine* 1994;12:783–90.
- Crowe JE, Bui PT, Siber GR, Elkins WR, Chanock RM, Murphy BR. Cold-passaged, temperature-sensitive mutants of human respiratory syncytial virus (RSV) are highly attenuated, immunogenic, and protective in seronegative chimpanzees, even when RSV antibodies are infused shortly before immunization. *Vaccine* 1995;13:847–55.
- Lewis FA, Rae ML, Lehman NI, Ferris AA. A syncytial virus associated with epidemic diseases of the lower respiratory tract in infants and young children. *Med J Aust* 1961;2:932–3.
- Friedewald WJ, Forsyth BR, Smith CB, Gharpure MA, Chanock RM. Low-temperature–grown RS virus in adult volunteers. *JAMA* 1968;204:690–4.



28. Erwin J, Landon JC. Spacious biocontainment suites for chimpanzees in infectious disease research. In: Erwin J, Landon JC, eds. Chimpanzee conservation and public health: environments for the future. Rockville, MD: Diagon/Bioqual, 1992:65-9.
29. Clements ML, Belshe RB, King J. Evaluation of bovine, cold-adapted human, and wild-type human parainfluenza type 3 viruses in adult volunteers and in chimpanzees. *J Clin Microbiol* 1991;29:1175-82.
30. Coates HV, Alling DW, Chanock RM. An antigenic analysis of respiratory syncytial virus isolates by a plaque reduction neutralization test. *Am J Epidemiol* 1966;83:299-313.
31. Karron RA, Wright PF, Hall SL, et al. A live attenuated bovine parainfluenza type 3 virus vaccine is safe, infectious, immunogenic, and phenotypically stable in infants and children. *J Infect Dis* 1995;171:1107-14.
32. Connors M, Collins PL, Firestone C, Sotnikov AV, Waitze A, Davis AR. Cotton rats previously immunized with a chimeric RSV FG glycoprotein develop enhanced pulmonary pathology when infected with RSV, a phenomenon not encountered following immunization with vaccinia-RSV recombinants or RSV. *Vaccine* 1992;10:475-84.
33. Connors M, Kulkarni AB, Firestone C, Holmes KL, Morse HCI, Sotnikov AV. Pulmonary histopathology induced by respiratory syncytial virus (RSV) challenge of formalin-inactivated RSV-immunized BALB/c mice is abrogated by depletion of CD4<sup>+</sup> T cells. *J Virol* 1992;66:7444-51.
34. Murphy BR, Webster RG. Orthomyxoviruses. In: Fields BN, Knipe DM, Howley PM, et al., eds. *Fields virology*. New York: Raven Press, 1996: 1313-51.
35. Karron RA, Wright PF, Newman FK, et al. A live human parainfluenza type 3 virus vaccine is attenuated and immunogenic in healthy infants and children. *J Infect Dis* 1995;172:1445-50.
36. Firestone CY, Whitehead SS, Collins PL, Murphy BR, Crowe JE Jr. Nucleotide sequence analysis of the respiratory syncytial virus subgroup A cold-passaged (*cp*) temperature sensitive (*ts*) *cpts*-248/404 live attenuated virus vaccine candidate. *Virology* 1996;225:419-22.
37. Groothuis JR, Simoes EAF, Levin MJ, et al. Prophylactic administration of respiratory syncytial virus immune globulin to high-risk infants and young children. *N Engl J Med* 1993;329:1524-30.
38. Piedra PA, Grace S, Jewell A, et al. Purified fusion protein vaccine protects against lower respiratory tract illness during respiratory syncytial virus season in children with cystic fibrosis. *Pediatr Infect Dis J* 1996;15:23-31.
39. Wright PF, Karzon DT. Live attenuated influenza vaccines. *Prog Med Virol* 1987;34:70-88.